(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 341 745 B1**

# (12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **14.12.94**

(51) Int. Cl.5: **C08B 15/00**, C08B 37/04, C08B 37/08, A61K 47/00, A61K 31/725, A61L 17/00

(21) Application number: **89108630.8**

(22) Date of filing: **12.05.89**

Divisional application 94108633.2 filed on 12/05/89.

The file contains technical information submitted after the application was filed and not included in this specification

## (54) Crosslinked carboxy polysaccharides.

(30) Priority: **13.05.88 IT 4796488**

(43) Date of publication of application:
**15.11.89 Bulletin 89/46**

(45) Publication of the grant of the patent:
**14.12.94 Bulletin 94/50**

(84) Designated Contracting States:
**AT BE CH DE ES FR GB GR IT LI LU NL SE**

(56) References cited:
**WO-A-87/07898**
**GB-A- 1 042 864**
**GB-A- 1 086 323**
**US-A- 4 521 594**

(73) Proprietor: **FIDIA S.p.A.**
**Via Ponte della Fabbrica 3-A**
**I-35031 Abano Terme (Padova) (IT)**

(72) Inventor: **della Valle, Francesco, Dr.**
**Via Cerato 14**
**Padova (IT)**
Inventor: **Romeo, Aurelio, Prof.**
**Viale-Ippocrate**
**I-93, Rome (IT)**

(74) Representative: **VOSSIUS & PARTNER**
**Postfach 86 07 67**
**D-81634 München (DE)**

EP 0 341 745 B1

## Description

The present invention concerns

A cross-linked hyaluronic acid product wherein the first portion of 1-100% of the carboxyl groups of said hyaluronic acid are cross-linked by ester bonding or lactonic bonding to hydroxyl groups of the same hyaluronic acid molecule and/or to hydroxyl groups of different hyaluronic acid molecules;
and the second portion of carboxyl groups if present of said hyaluronic acid are esterified with aliphatic, araliphatic, cycloaliphatic or heterocyclic alcohols,

wherein said alcohols of the aliphatic series have a maximum of 34 carbon atoms and may be substituted by one or two functional groups chosen from the group formed by amino, hydroxy, mercapto, aldehydo, ketal, carboxy, hydrocarbyl, and dihydrocarbylamino, ether, ester, thioester, acetal, ketal, carbamidic groups or carbamidic groups substituted by one or more alkyl groups, the hycrocarbyl radicals in these groups having a maximum of 6 functionally modified carbon atoms, and in which such alcohols of the aliphatic series may be interrupted in the carbon atom chain by heteroatoms chosen from the group formed by oxygen, sulfur and nitrogen, and wherein said alcohols of the araliphatic series have only one benzene residue and have an aliphatic chain with a maximum of 4 carbon atoms and wherein the benzene residue may be substituted by between 1 and 3 methyl or hydroxy groups, by halogen atoms, and wherein the aliphatic chain may be substituted by one or two functions chosen from the group consisting of free amino groups or mono- or diethyl groups or by pyrrolidine or piperidine groups, said alcohols of the cycloaliphatic or aliphatic-cycloaliphatic series are mono- or polycyclic hydrocarbons with a maximum of 34 carbon atoms, and said heterocyclic alcohols are mono- or polycyclic cycloaliphatic or aliphatic cycloaliphatic alcohols interrupted in their carbon atom chain or ring by one or more heteroatoms chosen from the group formed by nitrogen, oxygen and sulfur, or
wherein the alcohols of the cycloaliphatic or aliphatic cycloaliphatic series are derived from mono or polycyclic carbohydrates, and have a maximum of 34 carbon atoms, and may be unsubstituted and may contain one or more substituents, mentioned above for the aliphatic alcohols, or the aliphatic-cycloaliphatic polycyclic alcohols, sterols, cholic acids and steroids, or wherein the heterocyclic alcohols are derivatives of the abovesaid cycloaliphatic or aliphatic-cycloaliphatic alcohols, wherein the linear or cyclic chains are interrupted by one to three hetereo atoms chosen from the group formed by - O -, - S -, - N and -NH, as well as genins, digitoxigenin, gitoxigenin, digoxigenin, strophanthidin, tigogenin saponins and vitamin alcohols; and the third portion of carboxyl groups if present is either salified or in form of the free acid.

These "inner" esters of hyaluronic acids, in which there is no intervention by OH groups of other alcohols, can also be defined as "auto-crosslinked hyaluronic", since the formation of a mono- or polymolecular cross-link is the consequence of the abovementioned internal esterification. Hereafter, the new compounds of the present invention will be referred to by this definition. The adjective "cross-linked" refers to the crosswise connections between the carboxyls and hydroxyls of the hyaluronic molecules.

The new inner esters can be total or partial, depending on whether all or only part of the carboxy functions are esterified in the above manner. In the partial inner esters, further carboxy functions can be either totally or partially esterified with monovalent or polyvalent alcohols, thus forming "external" ester groups, and in the partial esters of both these ester groups the non-esterified carboxy functions may be free or salified with metals or organic bases.

Esterification between different hyaluronic molecules consequently increases their molecular weight, which can be roughly doubled or multiplied according to the number of molecules involved in the crosslinking. The degree of "polymerization" varies according to the conditions used in the preparation procedure described hereafter, such as temperature, reaction duration, but it may likewise depend on the hyaluronic acid to be crosslinked. Even though it is impossible to ascertain the ratio between the two types of ester bonds, an approximate representation can be made on the basis of the molecular weight, this being proportional to the number of molecules of the hyaluronic acid aggregate of the abovesaid bonds of intermolecular inner esters. Particularly important are the crosslinked products of the present invention, resulting from the fusion of two or three hyaluronic acid molecules, and products varying in their degree of "polymerization" in these terms. They can be obtained for example by means of the procedure used in the illustrative Examples.

The invention also concerns the use of the new inner esters, for example in the field of biodegradable plastic materials, to manufacture sanitary and surgical articles, in the cosmetic and pharmaceutical fields, in the food industry and in many other industrial fields.

Hyaluronic acid containing carboxy functions which serve as the basic starting materials to the new inner esters of the present invention are all those already known and described in literature, such as the natural ones of animal or vegetable origin, and synthetic derivatives of the same.

Also the external partial esters of hyaluronic acid, may serve as starting compounds.

The specific use of the new esters can be determined and depends upon the overall degree of esterification, inner and possibly external, that is the number of esterified carboxy functions, and also the number of salified groups, as well as the degree of aggregation ("polymerization") of the molecules involved in the process of esterification. These indeed are the factors which determine the solubility of the product and its viscous-elastic properties. Thus, for example, the total esters are practically insoluble in aqueous liquids and are suitable, due to their molecular structure, for use in the manufacture of plastic materials and as additives for such materials. The esters with medium or low degrees of esterification and their salts with inorganic or organic bases are more or less soluble in aqueous conditions and are suitable for the preparation of gels destined for various uses, in the cosmetic and pharmaceutical fields and in the medical-sanitary field in general.

The autocross-linked products of the present invention may possess all the carboxy functions in the form of an inner ester, or only an aliquot part of the same. In these partial inner esters the percentage of "cross-links" varies preferably between 1 and 60%, and especially between 5 and 30% of the number of carboxy groups in the hyaluronic acids.

The new inner esters of the present invention have become available because of the discovery of an original chemical procedure which is based on the activation of the carboxy groups by the addition of substances capable of inducing such activation. The unstable intermediate products obtained from the activation reaction separate spontaneously, either after the addition of catalysts and/or following a rise in temperature, forming the above mentioned inner ester bonds with hydroxyls of the same or other hyaluronic acid molecule. According to the degree of inner esterification desired, either all or an aliquot part of the carboxy functions are activated (the aliquot part being obtained by using an excess of activating substances or by suitable dosing methods).

The carboxy groups to be converted into inner ester groups can be activated starting from hyaluronic acids containing free carboxy groups, or, preferably, from hyaluronic acids containing salified carboxy groups, for example metal salts, preferably alkaline or alkaline earth metals, and above all with quaternary amonium salts, such as those descried hereafter. Salts with organic bases such as amines can however also be used as starting substances.

Methods for the activation of free or salified carboxy groups are per se known, particularly in the field of peptide synthesis, and those skilled in the art can easily determine which method is the most suitable, especially whether or not to use the starting substances in their free or salified form. Activation methods per se known for peptide synthesis procedures and useful in the preparation procedures of the present invention are described, for example, in Bodanszky, M., In search of new methods in peptide synthesis, Int. J. Peptide Protein Res. 25, 1985, 449-474; and Gross, E. et al., The Peptides, Analysis Synthesis, Biology, Academic Press, Inc., 1979, Vol. 1, Chapter 2. According to such procedures, a carboxyl component is activated, that is, a carboxyl component is converted to a reactive form. Such activation typically involves a reaction between an acid and an activating agent according to the scheme:

wherein X is on electron withdrawing moiety. Most activated derivatives of carboxylic acids, therefore, are mixed anhydrides, including in the broad sense also acid azides and acid chlorides which can be considered mixed anhydrides of hydrazoic acid and HCl as the activating agents. In addition, activation of a carboxyl component can be accomplished by the formation of intermediate "activated esters". These "activated esters" can be of various types, but particularly useful "activated esters" are those prepared by use of dicyclohexylcarbodiimide, p-nitrophenyl esters, trichlorophenyl esters, pentachlorophenyl esters, and O-acyl derivatives of hydroxylamines, particularly esters of N-hydroxysuccinimide.

All of these various types of activation procedures are useful in the preparation of the cross-linked hyaluronic acids of the invention, as all of these procedures can be characterized as importantly involving the reaction of a carboxyl group with an activating agent which essentially results in the formation of a substituent group that is easily reactive with a hydroxyl group so as to easily form the inner ester bonding characteristic of the products of the invention. The number of carboxy functions to be converted into inner esters is in proportion to the number of activated carboxy functions and this number depends on the quality of the activating agent used. In order to obtain total inner esters therefore, an excess of activating agents

should be used, while in the case of partial esters, the quantity of this agent should be dosed according to the degree of esterification desired.

The carboxy groups which are still free or salified after the cross-linking reaction according to the present invention can be exchanged in order to obtain opportune salts or can be esterified with the abovementioned monovalent or polyvalent alcohols thus obtaining mixed esters, partly cross-linked and partly externally esterified. Of course, partial esterification with alcohols can be effected before activation of part of the carboxy groups and subsequent conversion into inner esters, that is, the abovementioned hyaluronic esters can be used as starting substances.

The new procedure for the preparation of cross-linked hyaluronic acids is therefore characterized by treating a hyaluronic acid, having free or salified carboxy groups and possibly also carboxy groups esterified with mono- or polyvalent alcohols, with an agent which activates the carboxy function, possibly in the presence of in auxiliary agent favouring the formation of intermediate activated derivatives and/or a tertiary organic or inorganic base, exposing the mixture to heating or irradiation (particularly with UV light) and, if desired, esterification with mono- or polyvalent alcohols of the carboxy groups still free or salified in the hyaluronic acids thus obtained, and if desired, by salifying free carboxy groups or by freeing salified carboxy groups. Of the substances able to activate the carboxy group, the conventional ones described in literature can be used, for example those usually used in the synthesis of peptides, except however those which would have the effect of altering or destroying the molecular structure of the starting hyaluronic acid, such as those used for the formation of carboxyl halides. Preferred substances which lead to the formation of activated esters are those, such as, carbodiimides, dicyclohexylcarbodiimide, benzyl-isopropyl-carbodiimmide, benzyl-ethyl-carbodiimmide; ethoxyacetylene; Woodward's reagent (N-ethyl-5-phenylisoxazolium-3'-sulfonate), or halogen derivatives from aliphatic, cycloaliphatic or aromatic hydrocarbons, or from heterocyclic compounds with halogen made mobile by the presence of one or more activating groups, such as chloroacetonitryl and especially the salts of 2-chloro-N-alkypyridine, such as chloride of 2-chloro-N-methyl-pyridine or other alkyl derivatives with inferior alkyl groups, such as those with up to 6 carbon atoms. In the place of chloride derivatives, other halogen derivatives can of course be used, such as bromide derivatives.

This activation reaction can be carried out in organic solvents, especially aprotic solvents such as dialkylsulfoxides, dialkylcarboxylamides, such as in particular lower alkyl dialkylsulfoxides, particularly dimethylsulfoxide, polymethylene sulfoxides, such as tetramethylene sulfoxide, dialkyls or polymethylene sulfones, such as tetramethylene sulfone, sulfolane and lower alkyl dialkylamides of lower aliphatic acids in which the alkyl groups have a maximum of six carbon atoms, such as dimethyl or diethyl formamide or dimethyl or diethyl acetamide. If halogen derivatives are used as carboxyl-activating substances, especially in the form of salts, such as the above mentioned 2-chloro-N-methylpyridinium chloride, it is better to use a metal salt or a salt of the organic base of the starting hyaluronic acids, especially one of the quaternary ammonium salts described hereafter, such as tetrabutyl ammonium salt. These salts have the special advantage of being very soluble in the abovesaid organic solvents in which the crosslinking reaction is best effected, thus guaranteeing an excellent yield. It is advisable to add to the mixture a substance capable of subtracting acid, such as organic bases, carbonates, bicarbonates or alkaline or alkaline earth acetates, or organic bases and especially tertiary bases such as pyridine and its homologues, such as collidine, or aliphatic amine bases, such as triethylamine or N-methyl-piperazine

The use of quaternary ammonium salts represents a particulary advantageous procedure of the present invention and constitutes one of its main objectives. Such ammonium salts are well known and are prepared in the same way as other known salts. They derive from alkyls having preferably between 1 and 6 carbon atoms. It is preferable to use tetrabutyl ammonium salts. One variation in the procedure of the present invention in which quaternary ammonium salts are used, consists in reacting an alkaline salt, for example sodium or potassium salt, in the presence of catalyzing quantity of a quaternary ammonium salt, such as tetrabutylammonium iodide.

The substances which catalyze activation of the carboxy groups to be added to the activating agents are reported in literature and these too are preferably bases such as those mentioned previously. Thus, for example, when the carboxy groups are activated with isothiazoline salts it is preferable to add some triethylamine to the reaction mixture.

The reaction of formation of activated intermediates, such as and especially esters, is carried out at the temperature recommended in literature and this temperature can however be varied should circumstances require as can be easily determined by one skilled in the art. The formation of inner ester bonds can come about within a fairly wide temperature range, for example between 0° and 150°, preferably room temperature or slightly above, for example between 20° and 75°. Raising the temperature favours the formation of inner ester bonds, as does exposure to radiations of suitable wavelength, such as ultraviolet

rays.

In the produced hyaluronic acid crosslinked products, those remaining free carboxy groups or those in the form of salts can be partially or totally esterified with mono-or polyvalent alcohols, thus obtaining esters mixed with bonds which are in part internal and in part external. The alcohols used for this esterification correspond to those dealt with hereafter and from which the new mixed esters of the present invention are derived.

For esterification of the free or salified carboxy groups, known, conventional methods may be used, such as reaction between a carboxy salt, such as sodium salt, and an etherifying agent or the alcohols themselves in the presence of catalyzing substances, such as acid-type ion-exchangers. The known etherifying agents described in literature can be used, such as especially the esters of various inorganic acids or organic sulfonic acids, such as hydrogen acids, that is the hydrocarbyl halides such as methyl or ethyl iodide or neutral sulfates or hydrocarbyl acids, sulfites, carbonates, silicates, phosphites or hydrocarbyl sulphonates, such as methyl-, benzo-, or p-toluolsulfonate or methyl or ethyl chlorosulfonate. The reaction can take place in a suitable solvent, such as an alcohol, preferably the one corresponding to the alkyl group to be introduced into the carboxy group, but also nonpolar solvents such as ketones, ethers such as dioxane or aprotic solvents, such as dimethylsulfoxide. As a base, it is possible to use for example an alkaline or alkaline earth metal hydrate or magnesium or silver oxide or a basic salt of one of these metals, such as a carbonate and, of the organic bases, a tertiary nitrogenous base, such as pyridine or collidine. Instead of the base it is also possible to use a basic ion-exchanger. When starting from salts of partial hyaluronic esters, these may also be ammonium salts, such as ammonium or substituted ammonium salts.

According to one chemically original procedure described in the abovesaid European patent application No. 86305233.8, the external esters can be advantageously prepared by starting with quaternary ammonium salts with an etherifying agent in an aprotic solvent, such as dialkylsulfoxides, dialkylcarboxylamides, such as in particular lower alkyl dialkylsulfoxides with a maximum of 6 carbon atoms, particularly dimethylsulfoxide, and the lower alkyl dialkylamides of lower aliphatic acids, such as dimethyl or diethyl formamide or dimethyl or diethyl acetamide. Reaction should be effected preferably within a temperature range of between about 25° and 75°, for example at about 30°. Esterification is effected preferably by gradually adding the etherifying agent to the abovesaid ammonium salt dissolved in one of the solvents mentioned, for example in dimethylsulfoxide.

As alkylating agents it is possible to use those mentioned above, especially the alkyl halogens. As starting ammonium salts it is preferable to use lower ammonium tetraalkylates, since alkyl groups have preferably between 1 and 6 carbon atoms. It is best to use tetrabutyl ammonium salt. These quaternary ammonium salts can be prepared by reacting a metal salt of the acidic polysaccharide, in part internally esterified, preferably one of those mentioned above, especially sodium or potassium salt, in aqueous solution with a salified sulfonic resin with a quaternary ammonium base. The tetralkyl ammonium base of the polysaccharide ester can be obtained by freeze-drying the eluate. These starting salts are soluble in the above aprotic solvents, so that esterification according to this procedure is particularly easy and provides good yields. It is therefore only by following this procedure that the number of carboxy groups to be esterified can be exactly dosed.

One variation of this procedure consists in reacting potassium or sodium salt, suspended in a suitable solvent, such as dimethylsulfoxide, with a suitable alkylating agent in the presence of a catalyzing quantity of a quaternary ammonium salt, such as tetrabutyl ammonium iodide.

In the inner esters obtained according to the new procedure, the carboxy groups still left intact can be salified with organic or inorganic bases. The choice of bases for the formation of such salts is based on the intended use of the product. The inorganic salts are preferably those of alkaline metals, such as sodium or potassium salts or ammonium salts, cesium salts, salts of alkaline earth metals, such as calcium, magnesium or aluminum.

The salts of organic bases are especially those of aliphatic, araliphatic, cycloaliphatic or heterocyclic amines. The ammonium salts of this type may derive from therapeutically acceptable, but inactive, amines, or from amines with a therapeutic action. Of the former, special consideration should be given to aliphatic amines, for example, mono, di and trialkylamines, with alkyl groups with a maximum of 18 carbon atoms, or arylalkylamines with the same number of carbon atoms in the aliphatic part and where aryl means a benzene group possibly substituted by between 1 and 3 hydroxy groups. As therapeutically acceptable amines, but not active in themselves, cyclic amines are very suitable, such as alkylene amines with rings of between 4 and 6 carbon atoms, possibly interrupted in the ring by heteroatoms, such as oxygen, sulphur and nitrogen, such as piperidine, morpholine or piperazine, or may be substituted for example by amino or hydroxy functions, as in the case of aminoethanol, ethylene diamine or choline.

rays.

In the produced hyaluronic acid crosslinked products, those remaining free carboxy groups or those in the form of salts can be partially or totally esterified with mono-or polyvalent alcohols, thus obtaining esters mixed with bonds which are in part internal and in part external. The alcohols used for this esterification correspond to those dealt with hereafter and from which the new mixed esters of the present invention are derived.

For esterification of the free or salified carboxy groups, known, conventional methods may be used, such as reaction between a carboxy salt, such as sodium salt, and an etherifying agent or the alcohols themselves in the presence of catalyzing substances, such as acid-type ion-exchangers. The known etherifying agents described in literature can be used, such as especially the esters of various inorganic acids or organic sulfonic acids, such as hydrogen acids, that is the hydrocarbyl halides such as methyl or ethyl iodide or neutral sulfates or hydrocarbyl acids, sulfites, carbonates, silicates, phosphites or hydrocarbyl sulphonates, such as methyl-, benzo-, or p-toluolsulfonate or methyl or ethyl chlorosulfonate. The reaction can take place in a suitable solvent, such as an alcohol, preferably the one corresponding to the alkyl group to be introduced into the carboxy group, but also nonpolar solvents such as ketones, ethers such as dioxane or aprotic solvents, such as dimethylsulfoxide. As a base, it is possible to use for example an alkaline or alkaline earth metal hydrate or magnesium or silver oxide or a basic salt of one of these metals, such as a carbonate and, of the organic bases, a tertiary nitrogenous base, such as pyridine or collidine. Instead of the base it is also possible to use a basic ion-exchanger. When starting from salts of partial hyaluronic esters, these may also be ammonium salts, such as ammonium or substituted ammonium salts.

According to one chemically original procedure described in the abovesaid European patent application No. 86305233.8, the external esters can be advantageously prepared by starting with quaternary ammonium salts with an etherifying agent in an aprotic solvent, such as dialkylsulfoxides, dialkylcarboxylamides, such as in particular lower alkyl dialkylsulfoxides with a maximum of 6 carbon atoms, particularly dimethylsulfoxide, and the lower alkyl dialkylamides of lower aliphatic acids, such as dimethyl or diethyl formamide or dimethyl or diethyl acetamide. Reaction should be effected preferably within a temperature range of between about 25° and 75°, for example at about 30°. Esterification is effected preferably by gradually adding the etherifying agent to the abovesaid ammonium salt dissolved in one of the solvents mentioned, for example in dimethylsulfoxide.

As alkylating agents it is possible to use those mentioned above, especially the alkyl halogens. As starting ammonium salts it is preferable to use lower ammonium tetraalkylates, since alkyl groups have preferably between 1 and 6 carbon atoms. It is best to use tetrabutyl ammonium salt. These quaternary ammonium salts can be prepared by reacting a metal salt of the acidic polysaccharide, in part internally esterified, preferably one of those mentioned above, especially sodium or potassium salt, in aqueous solution with a salified sulfonic resin with a quaternary ammonium base. The tetralkyl ammonium base of the polysaccharide ester can be obtained by freeze-drying the eluate. These starting salts are soluble in the above aprotic solvents, so that esterification according to this procedure is particularly easy and provides good yields. It is therefore only by following this procedure that the number of carboxy groups to be esterified can be exactly dosed.

One variation of this procedure consists in reacting potassium or sodium salt, suspended in a suitable solvent, such as dimethylsulfoxide, with a suitable alkylating agent in the presence of a catalyzing quantity of a quaternary ammonium salt, such as tetrabutyl ammonium iodide.

In the inner esters obtained according to the new procedure, the carboxy groups still left intact can be salified with organic or inorganic bases. The choice of bases for the formation of such salts is based on the intended use of the product. The inorganic salts are preferably those of alkaline metals, such as sodium or potassium salts or ammonium salts, cesium salts, salts of alkaline earth metals, such as calcium, magnesium or aluminum.

The salts of organic bases are especially those of aliphatic, araliphatic, cycloaliphatic or heterocyclic amines. The ammonium salts of this type may derive from therapeutically acceptable, but inactive, amines, or from amines with a therapeutic action. Of the former, special consideration should be given to aliphatic amines, for example, mono, di and trialkylamines, with alkyl groups with a maximum of 18 carbon atoms, or arylalkylamines with the same number of carbon atoms in the aliphatic part and where aryl means a benzene group possibly substituted by between 1 and 3 hydroxy groups. As therapeutically acceptable amines, but not active in themselves, cyclic amines are very suitable, such as alkylene amines with rings of between 4 and 6 carbon atoms, possibly interrupted in the ring by heteroatoms, such as oxygen, sulphur and nitrogen, such as piperidine, morpholine or piperazine, or may be substituted for example by amino or hydroxy functions, as in the case of aminoethanol, ethylene diamine or choline.

Should the crosslinked hyaluronic acids of the present invention be intended for pharmacological and therapeutic uses, their vehicling functions can be put to good use (as explained hereafter) for therapeutically active amines, preparing the salts of such amines. These salts can therefore derive from all basic nitrogenous drugs, such as those of the following groups: alkaloids, peptides, phenothiazines, benzodiazepines, thioxanthenes, hormones, vitamins, anticonvulsivants, antipsychotics, antiemetics, anesthetics, hypnotics, anorexigenics, tranquilizers, muscle relaxants, coronary vasodilators, antineoplastics, antibiotics, antibacterials, antivirals, antimalarials, carbonic anhydrase inhibitors, nonsteroid antiinflammatory agents, vasoconstrictors, cholinergic agonists, cholinergic antagonists, adrenergic agonists, adrenergic antagonists, narcotic antagonists.

The salts can be prepared in a manner per se known in the art, for example by treating the crosslinked hyaluronic acids having a certain number of free carboxy functions, with the calculated quantity of base. However, salts can also be formed by double exchange; for example it is possible to obtain alkaline salts, such as sodium salt, treating a solution of quaternary ammonium salt of the crosslinked hyaluronic acids and/or partially esterified, with an aqueous solution of alkaline chloride, and isolating the alkaline salt present, for example by precipitation with a suitable solvent, such as a ketone, for example with acetone.

The cross-linked hyaluronic acids of the present invention may use, as starting substrate, any natural or synthetic hyaluronic acids substituted by carboxy groups, such as those corresponding to the above starting materials for the procedure of the invention.

Hyaluronic acid derivatives are of major importance compared to derivatives of other series of polysaccharides, due to the biological origin of the starting substrate, which permits the new crosslinked substances to be used in pharmaceutics, surgery and medicine in general.

The substrate of hyaluronic acid can be of any origin, such as acids extracted from the above natural starting materials, for example from cocks' combs. The preparation of these acids is described in literature: preferably, purified hyaluronic acids should be used. According to the invention, it is preferable to use hyaluronic acids constituting molecular fractions of the integral acids obtained directly by extraction of organic materials with a wide range of molecular weights, for example between 90%-80% and 0.2% of the molecular weight of the integral acid, preferably between 5% and 0.2%. These fractions can be obtained by various procedures described in literature, and that is with hydrolyzing, oxidizing or enzymatic chemical agents or physical procedures, for example mechanical or irradiation procedures, and often during the same purification procedures, primordial extracts may be formed. Separation and purification of the molecular fractions obtained comes about by means of known techniques, such as by molecular filtration. One purified HY fraction suitable to be used according to the invention is for example the one known as "noninflammatory-NIF-NaHA sodium hyaluronate", described by Balazs in the pamphlet "Healon" - A guide to its use in Ophthalmic Surgery - D. Miller & R. Stegmann, eds. John Wiley & Sons N.Y 81983: p.5.

Also particularly important as starting materials for the esters of the present invention are two purified fractions which can be obtained from hyaluronic acid, for example the one extracted from cocks' combs, known by the names of "Hyalastine" and "Hyalectin". The fraction Hyalastine has an average molecular weight of about 50,000 to 100,000 while the fraction Hyalectin has an average molecular weight of about 500,000 to 730,000. One combined fraction of these two fractions has also been isolated and characterized as having an average molecular weight of between about 250,000 and about 350,000. This combined fraction can be obtained with a yield of 80% of the total hyaluronic acid available in the particular starting material, while the fraction Hyalectin can be obtained with a yield of 30% and the fraction Hyalastine with a yield of 50% of the starting HY. The preparation of these fractions is described in the above-mentioned European patent publication No. 0138572A3.

Apart from hyaluronic acids themselves, it is possible to use their partial esters with mono or polyvalent alcohols as starting materials for the preparation of the new cross-linked products of the invention.

In the cross-linked hyaluronic acids of the invention which also have carboxy functions esterified with monovalent or polyvalent alcohols, whether these functions be present in the starting materials of the above mentioned procedure, or whether they be introduced at the end of the procedure, the alcohols may belong to the aliphatic, araliphatic, alicyclic or heterocyclic series.

The following description concerns the overall view of the above useful alcohols, on the understanding that the various groups and single compounds should be chosen on the basis of the particular hyaluronic substrates and their uses, as illustrated below. Thus, for example, one skilled in the art will know which alcohols are to be chosen for the cross-linked products intended for therapeutic and sanitary uses and which others are more suitable for the cross-linked products for use in the alimentary field or in the perfume industry or in the fields of resins and textiles.

Alcohols of the aliphatic series for use as esterifying components are those with a maximum of 34 carbon atoms, which can be saturated or unsaturated and which can possibly also be substituted by other

free functional or functionally modified groups, namely amino, hydroxyl, aldehydo, keto, mercapto, carboxy groups or by groups deriving from these, namely hydrocarbyl or dihydrocarbylamino groups (here and hereafter meaning by the term "hydrocarbyl" not only monovalent radicals of carbohydrates for example type $C_nH_{2n+1}$·, but also bivalent or trivalent radicals, such as "alkylenes" $C_nH_{2n}$ or "alkylideneS& $C_nH_{2n}$), ether or ester groups, acetal or ketal groups, thioether or thioester groups, and esterified carboxy groups or carbamidic and substituted carbamidic groups by one or two hydrocarbyl groups, by nitrile groups or halogens. Of the above groups containing hydrocarbyl radicals, these should preferably be inferior aliphatic radicals, such as alkylic, with a maximum of 6 carbon atoms. Such alcohols may then be interupted in the carbon atom chain by heteroatoms, such as atoms of oxygen, nitrogen and sulfur.

It is preferable to choose alcohols substituted with one or two of the abovesaid functional groups. Alcohols of the above group to be preferred for the purposes of the present invention are those with a maximum of 12 and especially 6 carbon atoms and in which the hydrocarbyl radicals in the abovesaid amino, ether,ester, thioether, thioester, acetal, ketal groups represent alkyl groups with a maximum of 4 carbon atoms, and also in the esterified carboxy groups or substituted carbamidic groups or hydrocarbyl groups are alkyls with the same number of carbon atoms, and in which the amino or carbamidic groups may be alkylene amine or alkylene carbamidic groups with a maximum of 8 carbon atoms. Of these alcohols special mention should be given to those which are saturated and unsubstituted such as methyl, ethyl, propyl, isopropyl alcohols, n-butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohols, pentyl, hexyl, octyl, nonyl and dodecyl alcohols and above all those with a linear chain, such as n-octyl and n-dodecyl alcohols. Of the substitued alcohols of this group, the follwing should be mentioned: bivalent alcohols such as ethylene glycol, propylene glycol, butylene glycol, trivalent alcohols such as glycerin, aldehyde alcohols such as tartronic alcohol, carboxy alcohols such as lactic acids, for example glycolic acid, malic acid, tartaric acids, citric acid, aminoalcohols, such as aminoethanol, aminopropanol, n-aminopropanol, n-aminobutanol and their dimethyl and diethyl derivatives in the amine function, choline, pyrrolidinylethanol, piperidinylethanol, piperazinylethanol and the corresponding derivatives of n-propyl or n-butyl alcohols, monothioethyleneglycol and its alkyl derivatives, for example the ethyl derivative in the mercapto function.

Of the higher aliphatic saturated alcohols, the following should be given as examples: cetyl alcohol and myricyl alcohol, but of special importance for the purposes of the present invention are the higher unsaturated alcohols with one or two double bonds, such as expecially those contained in many essential oils and having affinity with terpenes, such as citronellol, geraniol, nerol, nerolidol, linalool, farnesol, phytol. Of the lower unsaturated alcohols, the ones to be considered are allyl alcohol and propargyl alcohol.

The araliphatic alcohols, are those with only one benzene residue and in which the aliphatic chain has a maximum of 4 carbon atoms and in which the benzene residue may be substituted by between 1 and 3 methyl or hydroxy groups or by halogen atoms, especially by chlorine, bromine, iodine, and in which the aliphatic chain may be substituted by one or more functions chosen from the groups comprising free amino groups or mono or dimethyl or diethyl groups or by pyrrolidine or piperidine groups. Of these alcohols special mention should be given to benzyl alcohol and phenethyl alcohol.

Alcohols of the cycloaliphatic or aliphatic cycloaliphatic series may derive from mono or polycyclic carbohydrates, and have a maximum of 34 carbon atoms, may be unsubstituted and may contain one or more substituents, such as those mentioned above for the aliphatic alcohols. Of the alcohols derived from single-ringed cyclic carbohydrates, special mention should be given to those with a maximum of 12 carbon atoms, the rings having preferably between 5 and 7 carbon atoms, which may be substituted for example by between one and three lower alkyl groups, such as methyl, ethyl, propyl, or isopropyl groups. As alcohols specific to this group, cyclohexanol, cyclohexanediol, 1,2,3 cyclohexanetriol and 1,3,5 cyclohexanetriol (phloroglucitol), inositol, should be mentioned, as well as the alcohols deriving from p-menthane, such as carvomenthol, menthol, $\alpha$ and $\gamma$- terpineol, 1-terpinenol, 4-terpinenol and piperitol, or the mixture of these alcohols as "terpineol", 1,4-and 1,8-terpin. Of the alcohols deriving from carbohydrates with condensed rings, for example those of the thujane, pinane or camphane group, useful also are thujanol, sabinol, pinol hydrate, D and L-borneol and D and L-isoborneol.

Aliphatic-cycloaliphatic polycyclic alcohols to be used for the esters of the present invention are sterols, cholic acids and steroids, such as the sexual hormones and their synthetic analogues, and in particular corticosteroids and their derivatives. Thus it is possible to use for example: cholesterol, dihydrocholesterol, epidihydrocholesterol, coprostanol, epicoprostanol, sitosterol, stigmasterol, ergosterol, cholic acid, deoxycholic acid, lithocholic acid, estriol, estradiol, equilenin, equilin and their alkyl derivatives, as well as the ethynyl or propynyl derivatives in position 17, for example 17-$\alpha$ -ethynyl-estradiol or 7- $\alpha$-methyl-17-$\alpha$ -ethynyl-estradiol, pregnenolone, pregnanediol, testosterone and its derivatives, such as 17-$\alpha$ -methyl-testosterone, 1,2-dehydrotestosterone and 17- $\alpha$-methyl-1,2-dehydrotestosterone, alkynyl derivatives in

position 17 of testosterone and 1,2-dehydrotestosterone, such as 17- α-ethynyltestosterone, 17- α-propynyl-testosterone, norgestrel, hydroxyprogesterone, corticosterone, deoxycorticosterone, 19-nortestosterone, 19-nor-17- α-methyltestosterone and 19-nor-17- α-ethynyltestosterone, cortisone, hydrocortisone, prednisone, prednisolone, fludrocortisone, dexamethasone, betamethasone, paramethasone, flumethasone, fluocinolone, fluprednylidene, clobetasol, beclomethasone, aldosterone, desoxycorticosterone, alfaxalone, alfadolone, bolasterone.

Useful esterifying components for the esters of the present invention are genins, (aglycons) of cardioactive glycosides, such as digitoxigenin, gitoxigenin, digoxigenin, strophanthidin, tigogenin, saponins.

Other alcohols to be used according to the invention are vitamin alcohols such as axerophthol, vitamins $D_2$ and $D_3$, aneurine, lactoflavine, ascorbic acid, riboflavine, thiamine, pantothenic acid.

Heterocyclic alcohols may be considered to be derivatives of the abovesaid cycloaliphatic or aliphatic-cycloaliphatic alcohols, if their linear or cyclic chains are interrupted by one or more, for example between one and three ethero atoms chosen from the group formed by - O -, - S -, - N and -NH and in these there may be one or more unsaturated bonds for example double bonds, particulary between one and three, thus including also heterocyclic compounds with aromatic structures. The following are specific useful examples: furfuryl alcohol, alkaloids and derivatives such as atropine, scopolamine, cinchonine, cinchonidina, quinine, morphine, codeine, nalorphine, N-butylscopolammonium bromide, ajmaline; phenylethylamines such as ephedrine, isoproterenol, epinephrine; phenothiazine drugs such as perphenazine, pipothiazine, carphenazine, homofenazine, acetophenazine, fluphenazine, N-hydroxyethylpromethazine chloride; thioxanthene drugs such as flupenthizol and clopenthixol; anticonvulsivants such as meprophendiol, anti-psychotics such as opipramol; antiemetics such as oxypendil; analgesics such as carbetidine and phenoperidine and methadol; hypnotics such as etodroxizine; anorexics such as benzhydrol and diphemethoxidine; mild tranquilizers such as hydroxyzine; muscle relaxants such as cinnamedrine, diphylline, mephenesin, methocarbamol, chlorphenesin, 2,2-diethyl-1,3-propanediol, guaifenesin, idrocilamide; coronary vasodilators such as dipyridamole and oxyfedrine; adrenergic blockers such as propanolol, timolol, pindolol, bupranolol, atenolol, metoprolol, practolol; antineoplastics such as 6-azauridine, cytarabine, floxuridine; antibiotics such as chloramphenicol, thiamphenicol, erythromycin, oleandomycin, lincomycin; antivirals such as idoxuridine; peripheral vasodilators such as isonicotinyl alcohol; carbonic anhydrase inhibitors such as sulocarbilate; antiasthmatics and antiinflammatories such as tiaramide; sulfamides such as 2-p-sulfanylanilinoethanol.

While "inner" cross-linking of hyaluronic acids alone, without "external" esterification of the carboxy groups with alcohols of the aforesaid series, yields products which present properties similar to those of the starting products, but with the advantages mentioned previously, and may therefore be applied in all the fields in which the latter are used; simultaneous "external" esterification of the carboxy groups may prove useful in imparting to the hyaluronic acid properties specific to the alcohols themselves. In this case the crosslinked products act as a vehicle for the properties of the alcohols and in this manner can be put to good use in the pharmaceutical and medical fields. Thus, it is possible to prepare drugs containing cross-linked products according to the invention and therapeutically active alcohols, such as those listed above.

Salification, too, can have a double purpose, both in the manufacture of products in which the intrinsic properties of the basic hyaluronic acid are put to use, and in imparting to these the properties of the salifying bases, for example those with therapeutically active bases, for example those mentioned above.

The vehicling of a drug with the new cross-linked products can however also be achieved by the simple addition (physical mixture) of a drug and/or of a therapeutically active base to the hyaluronic acids. The present invention therefore also includes medicaments containing:

1. a pharmacologically active substance or an association of pharmacologically active substances and
2. a carrying vehicle comprising a cross-linked product of an hyaluronic acid according to the invention

Salts may be present in mixtures of this kind, should the following be chosen as component:

1) an organic base. Particulary important are associations of this type in which the component
2) is a cross-linked product having as its base hyaluronic acid or one of its esters.

The abovesaid medicaments may be in solid form, for example as freeze-dried powders containing only the two components 1) and 2) as a mixture or separately packed and this galenic form is especially suitable for topical use. Indeed such medicaments in solid form, on contact with the epithelium to be treated, form solutions which are more or less concentrated according to the nature of the particular epithelium and with the same characterisics as the solutions previously prepared in vitro and represent another aspect of the present invention. Such solutions are preferably in distilled water or in sterile physiological solutions and contain preferably no other pharmaceutical vehicle. The concentrations of these solutions may vary greatly, for example between 0.01 and 75%, both for the two separate components and for their mixtures. Preference should be given to solutions of a pronounced elastic viscous character, for example containing from 10% to 100% of the medicament or of each of the two components.

Particulary important are medicaments of this type, both in an anhydrous form (freeze-dried powder) or as solutions, either concentrated or diluted in water or saline, possibly with the addition of additives or auxiliary substances, such as particularly disinfectants or mineral salts acting as buffer or others, for ophthalmic use, based on cross-linked hyaluronic acid.

Among the medicaments of the type described here, preference should be given, as the case may be, to those with a degree of acidity suitable to the area in which they are to be applied, that is, with a physiologically tolerable pH. The pH may be adjusted by suitably regulating the quantity of hyaluronic acid, of its salts and of any basic or acid substances which may be present.

The degree of cross-linking and esterification depends firstly on the properties which are to be obtained in the various fields of application, for example a lesser or greater degree of lipophilia or hydrophilia in cases of therapeutic application. Usually, a high degree of cross-linking and esterification increases the lipophilic character of a substance and therefore diminishes its solubility in water. For a therapeutic use of the new cross-linked products it is important to regulate the degree of esterification in order to ensure, despite good and improved lipophilia compared to the basic hyaluronic acid or their salts, a sufficient degree of hydrosolubility. Naturally, the molecular size of the esterifying components should be considered, as it usually influences hydrosolubility in an inversely proportional manner.

The new cross-linked products, esterified with therapeutically active alcohols and/or salified with therapeutically active bases or the abovesaid medicaments containing them, are therapeutically more efficacious, and have a greater and/or longer-lasting effect (retard effect) as compared to the starting drugs.

Hyaluronic acid also constitutes however a very important substrate thanks to its own pharmaceutical action. The cross-linked products based on this polysaccharide, possibly also esterified with therapeutically inactive alcohols, have improved stability compared to hyaluronic acid itself and its esters. Such cross-linked products can be used for all known indications for the above compounds, for example hyaluronic acid itself, for example intraarticular injections with a lubricant action. As a result of the greater stability of the new cross-linked products with regard to hyaluronidase as compared to the free acid and to the esters, its action is greatly prolonged. The pharmacologically inert alcohols with which to esterify such cross-linked products of hyaluronic acid are preferably lower aliphatic alcohols with a maximum of 8 carbon atoms, especially saturated monovalent alcohols, such as ethanol, propyl alcohol, isopropyl alcohol, and n-butyl alcohol or isobutyl alcohol.

The cross-linked products based on hyaluronic acid are very suitable for cosmetic uses. Of the esters of these cross-linked products, important are those deriving from therapeutically inactive alcohols, such as for example saturated or unsaturated aliphatic alcohols, for example unsubstituted alcohols of this kind with a straight or ramified chain, for example with between 1 and 8 carbon atoms, such as those mentioned above. Particularly intersting are also unsaturated alcohols, for example with one or more double bonds, such as vinyl or allyl alcohols and their condensed derivatives, or polyvalent alcohols, such as glycerine. Also useful are aliphatic alcohols, for example those derived from cyclopentane or cyclohexane and their derivatives substituted by lower alkyl groups, for example alkyls with between 1 and 4 carbon atoms, especially by methyl groups. Particularly interesting are also esters with cycloaliphatic and aliphatic-cycloaliphatic alcohols derived from terpenes, such as those mentioned above and from therapeutically active alcohols, and which are also useful in cosmetics.

Extremely important is the use of cross-linked products based on hyaluronic acid for the manufacture of sanitary and surgical items. The esters of these cross-linked products are preferably those mentioned above for use in cosmetics.

The use of hyaluronic cross-linked products as vehicles for drugs intended for topical use is particularly useful in ophthalmology, where a particular compatibility is noted between the new products and the corneal epithelium, and therefore also excellent tolerability, with no sensitization effects. Furthermore, when the medicaments are administered in the form of concentrated solutions with elastic-viscous characteristics or in solid form, it is possible to obtain, on the corneal epithelium, homogenous and stable films which are perfectly transparent and adhering, which guarantee prolonged bioavailability of the drug and which therefore constitute excellent preparations with a retard effect. Such ophthalmic medicaments are particularly valuable in the veterinary field, considering that no chemotherapeutic specialities exist in this field, for example, veterinary specialities for ocular use containing chemotherapeutic components. As a result, preparations intended for humans are normally used and these do not always guarantee a specific range of action, nor they do allow for the particular conditions in which the treatment must be effected. This, for example, is the case of infective keratoconjunctivitis, pink eye or IBK, an infection which mainly affects cattle, sheep and goats.

The new cross-linked hyaluronic products and possibly medicaments of the type described above which contain them as component 2) may be applied in other fields too, and markedly in dermatology and

in diseases of the mucosa, for example of the mouth. Furthermore, they can be used to obtain a systemic effect thanks to transuctaneous riabsorption, for example in suppositories. All these applications are possible both in human and veterinary medicine. In human medicine the new medicaments are particularly suitable for pediatric use. The present invention includes in particular any one of these therapeutic applications.

Also objects of the present invention are pharmaceutical preparations containing one or more cross-linked hyaluronic acid products as defined above or associative medicaments containing them as component 2) also mentioned above. Apart from the therapeutically active substance or substances, such pharmaceutical preparations also contain the usual excipients and may be destined for oral, rectal, parenteral, subcutaneous, local or intradermal use. They are therefore in solid or semisolid form, for example pills, tablets, gelatinous capsules, capsules, suppositories, soft gelatin capsules. For parenteral and subcutaneous uses those forms intended for intramuscular or intradermal uses, or suitable for infusions or intravenous injections can be used, and can therefore be presented as solutions of the active compounds or as freeze-dried powders of the active compounds to be mixed with one or more pharmaceutically acceptable excipients or diluents, and which are suitable for the above uses being osmotically compatible with the physiological fluids. For local use, those preparations in the form of sprays should be considered, for example nasal sprays, creams and ointments for topical use or sticking plasters specially prepared for intradermal administration. Solubility of the cross-linked products in organic solvents with low boiling points makes them particularly suitable for the manufacture of "sprays".

The preparations of this invention can be administered to man or animal. They contain preferably between 0.01% and 10% of active component for the solutions, sprays, ointments and creams and between 1% and 100% and preferably between 15% and 50% of active compound for the solid form preparations. Dosages to be administered will depend on individual diagnoses, on the desired effect and on the chosen administration route. The daily dosages of these preparations can be deducted from those already used both for the basic hyaluronic acid for the corresponding cures, for example the cure for arthritis, for example in man or horse, and for the alcoholic component, in the case of esters, or of component 1) in the above medicaments, should these components represent the active principal whose action is to be exploited. Thus, for example, a cross-linked product of hyaluronic acid esterified even partially with cortisone, can be dosed according to its content of this steroid and to the usual dosage of the same in the known pharmaceutical preparations.

The preparation of salts according to the invention can be carried out in per se known procedures, by bringing into contact solutions either in aqueous suspensions or in organic solvents of the two components 1) and 2) and possibly of bases or basic salts of the above alkaline or alkaline earth metals or magnesium or alluminium in calculated quantities and isolating the salts in amorphous anhydrous form according to known techniques. It is possible for example to first prepare aqueous solutions of the two components 1) and 2), release such components from aqueous solutions of their salts with suitable ionic exchangers, mix the two solutions at a low temperature, for example between 0° and 20°, if the salt thus obtained is easily soluble in water it can be freeze-dried, while poorly soluble salts may be separated by centrifugation or filtration or decantation and possibly subsequently dried.

For these associated medicaments too, dosage is based on that of the active principles used singly and may therefore be easily determined by one skilled in the art, taking into consideration the dosages recommended for corresponding known drugs. In the cosmetic articles according to the invention, the cross-linked hyaluronic acid products and their salts are mixed with the excipients commonly used in the art and are for example those already listed above for pharmaceutical preparations. Above all, creams, ointments, lotions for topical use are used, in which the crosslinked hyaluronic acid or one of its salts can constitute the active cosmetic principle, possibly with the addition of other cosmetically active principles, such as for example steroids, for example pregnenolone, or one of the principles already reported. In such hyaluronic acids, the carboxy groups not used in cross-linking are preferably free or salified or are esterified with pharmacologically inactive alcohols, for example one of the lower aliphatic alcohols mentioned previously. The cosmetic articles can however also contain groups esterified with alcohols which have themselves a cosmetic action or an action which is auxillary to the same, such as for example disinfectant substances, sunshields, waterproofing or regenerating or antiwrinkle substances or odoriferous substances, especially perfumes. Such substances may however also be simply mixed with the cross-linked hyaluronic acid, thus constituting cosmetic compositions similar to the medicaments previously described in which the pharmaceutically active component 1) is substituted by a cosmetological factor. Use of the cosmetic preparations of the present invention in the perfume industry represents a great step forward in techniques, since it allows slow, constant and protracted release of the odorous principles.

An important object of the present invention is constituted by sanitary and surgical articles, by their manufacturing methods and by their use. These articles are for example similar to those already known and commercially available or described in literature, for example those with a hyaluronic acid base, for example inserts or ophthalmic lenses.

Surgical and sanitary articles of special importance are those which can be obtained from appropriate solutions of the cross-linked products in organic liquids which are capable of being made into films, sheets and threads to be used in surgery as auxillary or substitutive articles for the skin in cases of serious damage to this organ, such as burns, or as suture threads in surgery. The invention includes particularly these uses and a preparation procedure for these articles consisting in (a) forming a solution of the crosslinked hyaluronic acid or of one of its salts in an organic solvent; (b) making this solution into sheet or thread form; and (c) removing the organic solvent.

The formation of a solution of the crosslinked hyaluronic acid or of one of its salts is conducted in a suitable organic solvent, for example a ketone, an ester or an aprotic solvent such as an amide of a carboxy acid, especially a dialkylamide or of an aliphatic acid with between 1 and 5 carbon atoms and deriving from alkyl groups with between 1 and 6 carbon atoms, and above all from an organic sulfoxide, that is a dialkylsulfoxide with alkyl groups with a maximum of 6 carbon atoms, such as especially dimethylsulfoxide or diethylsulfoxide and also especially a fluorurate solvent with a low boiling point, such as especially hexafluoro-isopropanol.

Removing the organic solvent (c) is conducted by contact with another organic or aqueous solvent which must be mixable with the first solvent and in which the hyaluronic acid ester is insoluble, especially a lower aliphatic alcohol, for example ethyl alcohol (wet spinning), or, should a solvent with a not too high boiling point be used to prepare the solution of the hyaluronic acid derivative, in removing this same solvent by dry spinning, that is with a gas current and especially with suitably heated nitrogen. Dry-wet spinning can also be used with excellent results.

Particularly important are threads obtained with cross-linked products with a hyaluronic acid base, which can be used for the preparation of lints for the medication of wounds and in surgery. The use of such lints has the special advantage of being biodegradable to hyaluronic acid in the organism, by means of naturally existing enzymes. If cross-linked products containing also ester groups are used, these should be chosen from among those deriving from therapeutically acceptable alcohols, so that after enzymatic scission, apart from hyaluronic acid, innocuous alcohols are also formed, such as ethyl alcohol.

In the preparation of the abovesaid sanitary and surgical articles, it is possible also to include to advantage plasticizing materials in order to improve their mechanical characteristics, such as in the case of threads, to improve their resistance to tangles. Such plasticizers may be for example alkaline salts of fatty acids, for example sodium stearate, esters of organic acids with a high number of carbon atoms and the like.

Another application of hyaluronic cross-linked products where their biodegradability by esterases present in the organism is exploited, is represented by the preparation of capsules for subcutaneous implantation of medicaments or microcapsules by injection, for example by subcutaneous or intramuscular route. Up till now, for the application of subcutaneous medicaments designed to give slow release and therefore a retard effect, capsules made of silicon materials have been used, with the disadvantage that such capsules tend to migrate within the organism with no possibility of recovering them. Clearly, with the new hyaluronic derivatives this danger has been eliminated.

Of great importance is the preparation of microcapsules based on cross-linked hyaluronic products, eliminating the problems associated with their use, until now very limitated for the same reasons as those explained above and opening up a vast field of application wherever a retard effect by injective route is desired.

Another application in the fields of medicine and surgery of the cross-linked hyaluronic products is represented by the preparation of various solid inserts such as plates, discs, sheets, and the like to replace those currently in use which are made of metal or synthetic plastic material, wherever these inserts are destined for removal after a certain period of time. Preparations with an animal collagen base, being proteic by nature, often have unpleasant side effects such as inflammation or rejection. In the case of cross-linked hyaluronic products, even though they are made of animal and not human hyaluronic acid, this danger does not exist as there is no incompatibility between the polysaccharides of various animal species.

Another application regards their use in increasing and correcting defects in the soft tissues: for a long time now there has been an urgent call for safe and effective biomaterials with which to substitute lost or damaged soft tissues. Many materials have been used such as paraffin, teflon paste, silicone and bovine collagen to replace lost soft tissues. However, these materials were associated with undesirable and permanent changes in the skin, with in situ migration of implants and negative reactions. For this reason

there is a constant call in medicine for a versatile biomaterial. The cross-linked products of hyaluronic acid may be safely used to correct such defects of the soft tissues such as acne scars, postsurgical atropic irregularities, Mohs' chemosurgery, lacerated scars of the lip and old-age wrinkles.

Part of the applications in the fields of medicine and surgery of the new hyaluronic derivatives according to the present invention are preparations made of expanding material, especially in the form of sponges, for the medication of wounds or various lesions.

The above applications of the cross-linked products with a hyaluronic acid base represent the ideal solution for those sanitary and surgical articles which are intended to be introduced in one way or another into human or animal organisms or to be externally applied to the same.

With regard to the physical, pharmacological and therapeutic properties, the substantial equivalence between the hyaluronic acid cross-linked products of the present invention, possibly esterified with the abovesaid alcohols, and their salts, such as metal salts, it should be understood that the facts previously reported regarding the nonsalified products are true also of the salts.

The present invention also includes modifications in the preparation procedure for the new cross-linked products and their salts, in which a procedure is interrupted at any one stage or in which a procedure is begun with an intermediate compound and the remaining stages are carried out, or in which the starting products are formed in situ.

The invention is illustrated by the following illustrative examples, without these in any way limiting its scope.

Example 1:

PREPARATION OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

1% of carboxy groups used in internal esterification.
99% of carboxy groups salified with sodium.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °C, 0.01 g (0.1 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 0.026 g (0.1 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

3.97 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 2:

PREPARATION OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

5% of carboxy groups used in internal esterification.
95% of carboxy groups salified with sodium.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 85,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °C, 0.051 gr (0.5 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 0.128 gr (0.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times in 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

3.95 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 3:

PREPARATION OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

10% of carboxy groups used in internal esterification.
90% of carboxy groups salified with sodium.
6.21 g of HY tetrabutylammonium salt with a molecular weight of 620,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °C, 0.101 gr (1.0 mEq) of triethylamine is added and the resulting solution is agitated for 30 minutes.
A solution of 0.255 gr (1.0 mEq) of 2-chloro-1-methyl-pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.
A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times in 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.
3.93 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 4:

PREPARATION OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

25% of carboxy groups used in internal esterification.
75% of carboxy groups salified with sodium.
6.21 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°, 0.253 g (2.5 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.
A solution of 0.639 g (2.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.
A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times in 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.
3.85 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 5:

PREPARATION OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

50% of carboxy groups used in internal esterification.
50% of carboxy groups salified with sodium.
6.21 g of HY tetrabutylammonium salt with a molecular weight of 85,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°, 0.506 g (5.0 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 1.28 gr (5 mEq) of 2-chloro-1-methylpyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times in 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30 °.

3.65 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 6:

PREPARATION OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

75% of carboxy groups used in internal esterification.

25% of carboxy groups salified with sodium.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °, 0.759 gr (7.5 mEq) of triethylamine is added and the resulting solution is agitated for 30 minutes.

A solution of 1.92 gr (7.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times in 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30 °.

3.54 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 7:

PREPARATION OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

100% of carboxy groups used in internal esterification.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 70,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °, 1.012 gr (10 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 2.55 gr (10 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.

The resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is filtered and washed six times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30 °.

3.52 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 8:

PREPARATION OF THE PARTIAL ETHYL ESTER OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

25% of carboxy groups esterified with ethanol; 25% of carboxy groups used in internal esterification.

50% of carboxy groups salified with sodium.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°, 0.390 gr (2.5 mEq) of ethyl iodide are added and the solution is kept for 12 hours at 30°. 0.253 gr (2.5 mEq) of triethylamine are added and the solution is agitated for 30 minutes.

A solution of 0.639 g (2.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30°C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times in 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

3.84 grs of the title compound are obtained. Quantitative determination of the ethoxy groups is carried out according to the method of R.H. Cundiff and P.C. Markunas (Anal. Chem. 33, 1028-1930 (1961). Quantitative determination of the total ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 9:

PREPARATION OF THE PARTIAL ETHYL ESTER OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

50% of carboxy groups esterified with ethanol; 25% of carboxy groups used in internal esterification. 25% of carboxy groups salified with sodium.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 85,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°, 0.780 g (5.0 mEq) of ethyl iodide are added and the solution is kept for 12 hours at 30°. 0.253 gr (2.5 mEq) of triethylamine are added and the solution is agitated for 30 minutes.

A solution of 0.639 g (2.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30°C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times in 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

3.87 grs of the title compound are obtained. Quantitative determination of the ethoxy groups is carried out according to the method of R.H. Cundiff and P.C. Markunas (Anal. Chem. 33, 1028-1930 (1961). Quantitative determination of the total ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 10:

PREPARATION OF THE ETHYL ESTER OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

75% of carboxy groups esterified with ethanol; 25% of carboxy groups used in internal esterification.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°, 1.17 gr (7.5 mEq) of ethyl iodide are added and the solution is kept for 12 hours at 30°. 0.253 g (2.5 mEq) of triethylamine are added and the solution is agitated for 30 minutes.

A solution of 0.639 gr (2.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30°C.

The resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is filtered and washed five times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

3.91 grs of the title compound are obtained. Determination of the ethoxy groups is carried out according to the method of R.H. Cundiff and P.C. Markunas (Anal. Chem. 33, 1028-1930 (1961). Quantitative

determination of the total ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 11:

PREPARATION OF THE PARTIAL CORTISONE ESTER (C21) OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

20% of carboxy groups esterified with cortisone.
25% of carboxy groups used in internal esterification.
55% of carboxy groups salified with sodium.

6.21 gr of HY tetrabutylammonium salt with a molecular weight of 70,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °C. 0.85 gr (2 mEq) 21-bromo-4-pregnene- 17-α -ol-3, 11, 20-trion and the resulting solution is kept for 24 hours at 30 °C. 0.253 gr (2.5 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 0.639 g (2.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

4.5 grs of the title compound are obtained. Quantitative determination of cortisone, mild alkaline hydrolysis with a hydroalcoholic solution of $Na_2CO_3$ and extraction with chloroform, is carried out according to B.P.

Quantitative determination of the total ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 12:

PREPARATION OF THE MIXED ETHANOL AND CORTISONE PARTIAL ESTER (C21) OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

20% of carboxy groups esterified with cortisone (C21).
25% of carboxy groups esterified with ethanol.
25% of carboxy groups used in internal esterification.
30% of carboxy groups salified with sodium.

6.21 gr of HY tetrabutylammonium salt with a molecular weight of 85.000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25`C. 0.39 gr (2.5 mEq) of ethyl iodide are added and the resulting solution is kept at 30° for 12 hours. 0.85 gr (2 mEq) of 21-bromo-4-pregnene-17-α -ol-3,11, 20-trion are added and the resulting solution is kept at 30`C for 24 hours. 0.253 gr (2.5 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 0.639 g (2.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a period of 1 hour and the mixture is kept for 15 hours at 30 °C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

4.41 grs of the title compound are obtained. Quantitative determination of cortisone, mild alkaline hydrolysis with a hydroalcoholic solution of $Na_2CO_3$ and extraction with chloroform, is carried out according to B.P.

Quantitative determination of the ethoxy groups is carried out according to the method of R.H. Cundiff and P.C. Markunas (Anal. Chem. 33, 1028-1030 (1961). Quantitative determination of the total ester groups

is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 13:

PREPARATION OF THE MIXED ETHANOL AND CORTISONE ESTER (C21) OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

20% of carboxy groups esterified with cortisone (C21).
70% of carboxy groups esterified with ethanol.
10% of carboxy groups used in internal esterification.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °C. 1.09 g (7 mEq) of ethyl iodide are added and the resulting solution is kept at 30° for 12 hours. 0.85 gr (2 mEq) of 21-bromo-4-pregnene- 17- α-ol-3,11, 20-trion and the resulting solution is kept at 30 °C for 24 hours. 0.101 gr (1.0 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 0.255 g (1.0 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30 °C.

The resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed five times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30 °C.

4.58 grs of the title compound are obtained. Quantitative determination of cortisone, mild alkaline hydrolysis with a hydroalcoholic solution of $Na_2CO_3$ and extraction with chloroform, is carried out according to B.P.

Quantitative determination of the ethoxy groups is carried out according to the method of R.H. Cundiff and P.C. Markunas (Anal. Chem. 33, 1028-1030 (1961). Quantitative determination of the total ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example 14:

PREPARATION OF THE PARTIAL TETRABUTYLAMMONIUM SALT OF HYALURONIC ACID (HY)

Product description:

25% of carboxyls salified with tetrabutylammonium.
75% of carboxyls in acid form.

4.0 gr of HY sodium salt with a molecular weight of 170,000, corresponding to 10 mEq of a monomeric unit, are solubilized in 400 ml of distilled $H_2O$, and then passed through a thermostatic column at 5 °C, containing 15 ml of sulfonic resin (Dowex 50x8) in $H^+$ form. The sodium-free eluate, kept at a temperature of 5 °C, is added to 25 ml of a solution of 0.1 M of tetrabutylammonium hydroxide, while under constant agitation.

The resulting solution is frozen and freeze-dried.

Example 15:

PREPARATION OF CROSS-LINKED HYALURONIC ACID SALT WITH CARTEOLOL

Product description:

25% of carboxy groups used in internal esterification.
75% of carboxy groups with carteolol.

4.39 gr of partial tetrabutylammonium salt (25%) of hyaluronic acid corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°, 0.253 gr (2.5 mEq) of triethylamine is added and the resulting solution is agitated for 30 minutes.

A solution of 0.639 g (2.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30°.

The resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed five times with 100 ml di acetone and lastly vacuum-dried for 24 hours at 30°.

The precipitate is suspended in 400 ml of distilled water and cooled to 5°C.

2.19 gr (7.5 mEq) of basic carteolol are added and the whole is agitated for 30 minutes. The resulting mixture is freeze-dried.

5.8 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Analytical determination of carteolol is carried out according to the method of S.Y. Chu [J. Pharmac. Sci. 67, 1623 (1978)].

Example: 16

PREPARATION WITH KANAMYCIN OF THE SALT OF A CROSS-LINKED HYALURONIC ACID

Product description:

25% of carboxy groups used in internal esterification.

75% of carboxy groups with kanamycin.

4.39 gr of partial tetrabutylammonium salt (25%) of hyaluronic acid corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°, 0.253 gr (2.5 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 0.639 g (2.5 mEq) of 2-chloro-1-methyl-pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30°.

The resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed five times with 100 ml di acetone and lastly vacuum-dried for 24 hours at 30°.

The precipitate is suspended in 400 ml of distilled water and cooled to 5°C after which a solution obtained by solubilizing 1.1 gr of Kanamycin sulfate (7.5 mEq) in 25 ml of distilled $H_2O$ and eluting in a column containing 15 ml of quaternary ammonium resin (Dowex 1x8) OH- form is added, while agitation is maintained for 30 minutes. The resulting mixture is freeze-dried.

4.6 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Microbiological quantitative determination of Kanamycin is carried out on B. subtilis 6633 in comparison to standard Kanamycin.

Example: 17

PREPARATION WITH AMIKACIN OF A CROSS-LINKED HYALURONIC ACID SALT

Product description:

25% of carboxy groups used in internal esterification.

75% of carboxy groups with amikacin.

4.39 gr of partial tetrabutylammonium salt (25%) of hyaluronic acid corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°, 0.253 gr (2.5 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 0.639 gr (2.5 mEq) of 2-chloro-1-methyl-piridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30°.

The resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed five times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

The precipitate is suspended in 400 ml of distilled water and cooled to 5°C.

1.1 gr (7.5 mEq) of basic amikacin are added while under constant agitation for 30 minutes. The resulting mixture is freeze-dried.

4.8 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Quantitative determination of amikacin is carried out microbiologically on S. aureus 29737, compared to standard Amikacin.

Example: 18

PREPARATION OF THE PARTIAL ETHYL ESTER OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

50% of carboxy groups esterified with ethanol.

10% of carboxy groups used in internal esterification.

40% of carboxy groups salified with sodium.

6.21 gr of HY tetrabutylammonium salt with a molecular weight of 85.000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °C, 0.780 gr (5.0 mEq) of ethyl iodide are added and the solution is kept for 12 hours at 30°. 0.118 gr (1 mEq) of pyridine chloride are added and the resulting solution is agitated for 30 minutes.

A solution of 0.16 g (1 mEq) of N-benzyl-N'-ethyl carbodiimmide in 20 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 45 hours at 30°.

A solution is then added which is formed of 100 ml of water and 2.5 of sodium chloride and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times with 100 ml of acetone/$H_2O$ 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

3.85 grs of the title compound are obtained. Quantitative determination of the ethoxy groups is carried out according to the method of R.H. Cundiff and P.C. Markunas (Anal. Chem. 33, 1028-1930 (1961). Quantitative determination of the total ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

Example: 19

PREPARATION OF CROSS-LINKED HYALURONIC ACID (HY)

Product description:

10% of carboxy groups used in internal esterification.

90% of carboxy groups salified with sodium.

6.21 gr of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25 °C, 0.118 gr (1 mEq) of pyridine chloride are added and the resulting solution is agitated for 30 minutes.

A solution of 0.16 g (1 mEq) of N-benzyl-N'-ethyl carbodiimmide in 20 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept at a temperature of 30° for 45 hours.

A solution made up of 100 ml of water and 2.5 of sodium chloride is added and the resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times with 100 ml of acetone/$H_2O$ 5:1 and three times with 100 ml of acetone finally vacuum-dried for 24 hours at a temperature of 30°.

3.9 grs of the title compound are obtained. Quantitative determination of the total ester groups is carried out according to the saponification method described on pp 169-172 of "Quantitative Organic Analysis Via Functional Groups" 4th Edition John Wiley and Sons Publication.

The above preparation examples are only exemplary of the various cross-linked hyaluronic acids according to the invention. Other specifically desired products can be prepared by following the above described procedures, but substituting as appropriate other starting materials and/or reactants to result in the desired cross-linked product.

As discussed above, the new hyaluronic esters of the invention are useful for the preparation of pharmaceutical formulations and now medical articles. The following are particular exemplary pharmaceutical preparations according to the invention.

Formulation 1 - Collirium containing cortisone of which 100 ml contain:

| | |
|---|---|
| - partial and mixed ester of hyaluronic acid with cortisone and ethanol (Ex. 32) | gr. 0.300 |
| - ethyl p. hydroxybenzoate | gr. 0.010 |
| - methyl p. hydroxybenzoate | gr. 0.050 |
| - sodium chloride | gr. 0.900 |
| - water for injectable preparation/q.b.a. | ml. 100 |

Formulation 2 - Cream containing a partial ester of hyaluronic acid with ethanol of which 100 gr. contain:

| | |
|---|---|
| - partial ester of hyaluronic acid with ethanol (Ex. 9) | gr. 0.2 |
| - polyethyleneglycol monostearate 400 | gr. 10.000 |
| - Cetiol V | gr. 5.000 |
| - Lanette SX | gr. 2.000 |
| - Paraoxybenzoate of methyl | gr. 0.075 |
| - Paraoxybenzoate of propyl | gr. 0.050 |
| - Sodium dihydroacetate | gr. 0.100 |
| - Glycerine F.U. | gr. 1.500 |
| - Sorbitol 70 | gr. 1.500 |
| - Test cream | gr. 0.050 |
| - Water for injectable preparation/q.b.a. | gr. 100.00 |

## Claims

1. A cross-linked hyaluronic acid product wherein the first portion of 1-100% of the carboxyl groups of said hyaluronic acid are cross-linked by ester bonding or lactonic bonding to hydroxyl groups of the same hyaluronic acid molecule and/or to hydroxyl groups of different hyaluronic acid molecules;
and the second portion of carboxyl groups if present of said hyaluronic acid are esterified with aliphatic, araliphatic, cycloaliphatic or heterocyclic alcohols,

wherein said alcohols of the aliphatic series have a maximum of 34 carbon atoms and may be substituted by one or two functional groups chosen from the group formed by amino, hydroxy, mercapto, aldehydo, ketal, carboxy, hydrocarbyl, and dihydrocarbylamino, ether, ester, thioester, acetal, ketal, carbamidic groups or carbamidic groups substituted by one or more alkyl groups, the hycrocarbyl radicals in these groups having a maximum of 6 functionally modified carbon atoms, and in which such alcohols of the aliphatic series may be interrupted in the carbon atom chain by heteroatoms chosen from the group formed by oxygen, sulfur and nitrogen, and wherein said alcohols of the araliphatic series have only one benzene residue and have an aliphatic chain with a maximum of 4 carbon atoms and wherein the benzene residue may be substituted by between 1 and 3 methyl or hydroxy groups, by halogen atoms, and wherein the aliphatic chain may be substituted by one or two functions chosen from the group consisting of free amino groups or mono- or diethyl groups or by pyrrolidine or piperidine groups, said alcohols of the cycloaliphatic or aliphatic-cycloaliphatic series are mono- or polycyclic hydrocarbons with a maximum of 34 carbon atoms, and said heterocyclic alcohols are mono- or polycyclic cycloaliphatic or aliphatic cycloaliphatic alcohols interrupted in their carbon atom chain or ring by one or more heteroatoms chosen from the group formed by nitrogen, oxygen and sulfur, or

wherein the alcohols of the cycloaliphatic or aliphatic cycloaliphatic series are derived from mono or polycyclic carbohydrates, and have a maximum of 34 carbon atoms, and may be unsubstituted and may contain one or more substituents, mentioned above for the aliphatic alcohols, or the aliphatic-cycloaliphatic polycyclic alcohols sterols, cholic acids and steroids, or wherein the heterocyclic alcohols are derivatives of the abovesaid cycloaliphatic or aliphatic-cycloaliphatic alcohols, wherein the linear or cyclic chains are interrupted by one to three hetereo atoms chosen from the group formed by - O -, - S -, - N and -NH, as well as genins, digitoxigenin, gitoxigenin, digoxigenin, strophanthidin, tigogenin

saponins and vitamin alcohols; and the third portion of carboxyl groups if present is either salified or in form of the free acid.

2. The cross-linked hyaluronic acid product according to claim 1, wherein each of the carboxyl groups of said hyaluronic acid are ester bonded to a hydroxyl group.

3. The cross-linked hyaluronic acid product according to claim 1, wherein between 1% and 60% of the carboxyl groups of said hyaluronic acid are cross-linked to a hydroxyl group.

4. The cross-linked hyaluronic acid product according to anyone of claims 1 to 3, wherein the percentage of carboxyl groups involved in crosslinking ranges between 15% and 30%.

5. The cross-linked hyaluronic acid product according to claims 1-4, wherein said alcohol is an alcohol with a maximum of 32 carbon atoms and, in the case of alcohols substituted by functional groups, the hydrocarbyl radicals of the amine groups, ether, ester, thioether, thioester, acetal, ketal, represent alkyl groups with a maximum of 4 carbon atoms and in the esterified carboxy groups and in the substituted carbamidic groups the hydrocarbyl groups are alkyl groups with the same number of carbon atoms, and in which the substituted amino or carbamidic groups may also be alkyleneamino or alkylenecarbamidic groups with a maximum of 8 carbon atoms.

6. The cross-linked hyaluronic acid product according to claim 5, wherein said alcohol is ethyl, propyl, isopropyl, N-butyl, isobutyl, tert-butyl alcohols, an amyl, pentyl, hexyl or octyl alcohol, ethyleneglycol, propyleneglycol, butyleneglycol, glycerin, tartronic alcohol, lactic acid, glycolic acid, malic acid, a tartaric acid or citric acid.

7. The cross-linked hyaluronic acid product according to claims 1-4, wherein said heterocyclic alcohols are selected from the group consisting of alkaloids, phenylethylamines, phenothiazine drugs, thioxanthene drugs, sulfamidics, meprophendiol, opipramol, oxypendil; carbetidine and phenoperidine and methadol; etodroxizine; benzhydrol and diphemethoxidine; hydroxyzine; cinnamedrine, diphylline, mephenesin, methocarbamol, chlorphenesin, 2,2-diethyl-1,3-propanediol, guaifenesin, idrocilamide; dipyridamole and oxyfedrine; propanolol, timolol, pindolol, bupranolol, atenolol, metoprolol, practolol; 6-azauridine, cytarabine, floxuridine; chloramphenicol, thiamphenicol, erythromycin, oleandomycin, lincomycin; idoxuridine; isonicotinyl alcohol; sulocarbilate and tiaramide.

8. A salt of a cross-linked hyaluronic acid product according to anyone of claims 1 to 7, with an alkaline or alkaline earth metal, magnesium, aluminum or an amine.

9. A salt according to claim 8, with sodium or ammonium.

10. A salt according to claim 8, wherein said amine is a therapeutically acceptable base.

11. A salt according to claim 8, wherein said amine is a therapeutically active base.

12. A salt according to claim 8, wherein said amine is pharmacologically inactive and is selected from the group consisting of mono-, di- and tri-alkylamines with a maximum of 18 carbon atoms, arylalkylamines with a maximum of 18 carbon atoms in the aliphatic part and with a benzene group as an aromatic part, optionally substituted by between 1 and 3 methyl groups or halogen atoms or hydroxyl groups, alkyleneamines with cycles of between 4 and 6 carbon atoms optionally interrupted in the cycle by heteroatoms chosen from the group consisting of O and S, and amines of all these types substituted by amino or hydroxy functions.

13. A compound according to claim 1, selected from the group consisting of:
- hyaluronic acid cross-linked to an extent of 1% of the carboxy groups and salified with sodium to an extent of 99%;
- hyaluronic acid cross-linked to an extent of 5% of the carboxy groups and salified with sodium to an extent of 95%;
- hyaluronic acid cross-linked to an extent of 10% of the carboxy groups and salified with sodium to an extent of 90%;

- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups and salified with sodium to an extent of 75%;
- hyaluronic acid cross-linked to an extent of 50% of the carboxy groups and salified with sodium to an extent of 50%;
- hyaluronic acid cross-linked to an extent of 75% of the carboxy groups and salified with sodium to an extent of 25%;
- hyaluronic acid cross-linked to an extent of 100% of the carboxy groups;
- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups, esterified to an extent of 25% with ethanol and salified with sodium to an extent of 50%;
- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups, esterified to an extent of 50% with ethanol and salified with sodium to an extent of 25%; and
- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups and esterified with ethanol to an extent of 75%.

**14.** A compound according to claim 11 selected from the group consisting of:

- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups, esterified to an extent of 20% with cortisone and salified with sodium to an extent of 55%;
- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups, esterified with cortisone to an extent of 20% and with ethanol to an extent of 25% and salified with sodium to an extent of 30%;
- hyaluronic acid cross-linked to an extent of 10% of the carboxy groups, esterified with cortisone to an extent of 20% and with ethanol to an extent of 70%;
- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups and salified with carteololo to an extent of 75%;
- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups and salified to an extent of 75% with kanamycin; and
- hyaluronic acid cross-linked to an extent of 25% of the carboxy groups and salified with amikacin to an extent of 75%.

**15.** A pharmaceutical composition comprising as an active ingredient a compound according to claim 11 together with an excipient.

**16.** A medicament comprising:

(1) a pharmacologically active substance or a mixture of pharmacologically active substances; and

(2) a vehicle comprised of a cross-linked hyaluronic acid product according to anyone of claims 1 to 4.

**17.** A medicament according to claim 16, in which component (1) is a substance for oral, parenteral or topical use.

**18.** A medicament according to claim 17, wherein the component (1) is an anesthetic, analgesic, antiinflammatory, vasocontrictory antibiotic/antibacterial or antiviral agent.

**19.** A cosmetic article containing a cross-linked hyaluronic acid product according to anyone of claims 1 to 4.

**20.** A sanitary or surgical article containing a hyaluronic acid product according to anyone of claims 1 to 4.

**21.** A sanitary or surgical article according to claim 20, comprised of threads or films of a cross-linked hyaluronic acid product.

**22.** A sanitary or surgical article according to claim 21, comprised of capsules for the subcutaneous implantation of medicaments.

**23.** A sanitary or surgical article according to claim 21, comprised of microcapsules for subcutaneous, intramuscular or intravenous injection.

24. A sanitary or surgical article according to claim 20, comprised of sponges for the medication of wounds and lesions.

25. A process for the preparation of cross-linked hyaluronic acid products as defined in claims 1-14 which comprises:
(a) treating hyaluronic acid with an activating agent selected from those generally used in peptide synthesis or 2-halogen-N-$C_1$-$C_6$ alkyl-pyridine or chloroacetonitrile to activate carboxy groups in said hyaluronic acid to form intermediate activated hyaluronic acid derivatives; and
(b) subjecting said intermediate activated hyaluronic derivatives to heat or irradiation to produce cross-linked hyaluronic acid whereby the synthesis is carried out in an aprotic solvent at a temperature of 0-150 ° C.

26. A process according to claim 25, wherein at least a portion of the carboxy groups in said hyaluronic acid are salified.

27. A process according to claim 26, wherein said at least a portion of carboxy groups are salified with an alkaline or alkaline earth metal, or with a quaternary ammonium.

28. A process according to claim 25, wherein said treatment with an activating agent is performed in the presence of a catalyst.

29. A process according to any of claims 25-28, wherein said activating agent is a carbodiimide, ethoxyacetylene, Woodward's reagent, or chloroacetonitryl.

30. A process according to any of claims 25-28, wherein said activating agent is a 2-halogen-N-alkylpyridinium salt, in which the halogen is selected from the group consisting of chlorine and bromine and the alkyl has a maximum of 6 carbon atoms.

31. A process according to claim 30, wherein said activating agent is a chloride of 2-chloro-N-methyl-pyridine and reacts with a tetrabutyl-ammonium salt of the hyaluronic acid in the presence of a tertiary amine base.

32. A process according to claim 25, wherein said organic involving the aprotic solvent is a dialkylsulfoxide or a dialkylamide of a lower aliphatic alcohol with an alkyl having a maximum of 6 carbon atoms.

33. A process according to claim 25, wherein dimethylsulfoxide is used as said solvent.

34. A process according to claim 25 in which the reaction is carried out at room temperature.

35. A process according to any one of claims 25-34, wherein subsequent to said cross-linking reaction, at least a portion of any remaining free carboxyl groups in said cross-linked hyaluronic acid are salified or esterified with a mono- or polyvalent alcohol.

**Patentansprüche**

1. Vernetztes Hyaluronsäureprodukt, wobei ein erster Anteil von 1 bis 100 % Carboxylgruppen der Hyaluronsäure durch Esterbindung oder Lactonbindung mit Hydroxylgruppen des gleichen Hyaluronsäuremoleküls und/oder Hydroxylgruppen von unterschiedlichen Hyaluronsäuremolekülen verknüpft sind;
und ein zweiter Anteil der Carboxylgruppen der Hyaluronsäure, falls vorhanden, der Hyaluronsäure mit aliphatischen, araliphatischen, cycloaliphatischen oder heterocyclischen Alkoholen verestert ist, wobei die Alkohole der aliphatischen Reihe höchstens 34 Kohlenstoffatome aufweisen und substituiert sein können mit einer oder zwei funktionellen Gruppen, ausgewählt aus Amino-, Hydroxy-, Mercapto-, Aldehyd-, Ketal-, Carboxy-, Kohlenwasserstoff- und Dikohlenwasserstoffamino-, Ether-, Ester-, Thioester-, Acetal-, Ketal-, Carbamidogruppen oder Carbamidogruppen, die mit einem oder mehreren Alkylresten substituiert sind, wobei die Kohlenwasserstoffreste in diesen Gruppen höchstens 6 funktionell modifizierte Kohlenstoffatome aufweisen, und wobei die Alkohole der aliphatischen Reihe in der Kohlenstoffkette durch Heteroatome unterbrochen sein können, ausgewählt aus Sauerstoff, Schwefel